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FIRST TRIMESTER SCREENING OF HYPERTENSIVE DISORDERS OF PLACENTAL AND MATERNOGENIC ORIGIN

SSD MED/40

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Introduction

Hypertensive disorders in pregnancy represent a great dilemma for the clinicians. The incidence and the complications, both for mother and fetus, are high and occasionally severe.

Preeclampsia (PE) is the leading cause to perinatal mortality and morbidity[1]. Affecting the outcome of 2-8% of pregnancies, along with the other hypertensive disorders of pregnancy, it is a major contributor to maternal mortality everywhere[2]. Maternal death caused by hypertensive disorders represents 26% of the total in Latin America and the Caribbean, 9% in Africa and Asia[3]. The incidence in USA and Europe has risen maybe for the increased incidence of risk factors: chronic hypertension, obesity and diabetes[4]. Other hypertensive disorders in pregnancy are pre-existing hypertension and gestational hypertension. In the reproductive years, chronic hypertension is a rare disease, while gestational hypertension and preeclampsia constitute the bulk of hypertensive disorders in pregnancy.

These pregnancy complications not only alter the immediate outcomes of pregnancy at the time of delivery, but also the long-term cardiovascular health of the affected women and children. For example, a history of preeclampsia increases a female's risk of myocardial infarction, stroke or diabetes mellitus by two to eight folds over the next two decades[5]. Furthermore, such women have also been shown to have increased risk for renal diseases, such as, focal segmental glomerulosclerosis and microalbuminuria[6]. Moreover, newborns born after a pregnancy complicated by preeclampsia have a two to eightfold increased risk for hypertension, cardiovascular disease, diabetes mellitus or renal disease as adults[7]. The pathogenesis is unclear: maybe these conditions are related to epigenetic modification, postnatal growth acceleration, permanent alteration caused by fetal malnutrition[8].

Hypertensive disorders in pregnancy

Pregnancy induced hypertensive disease is a multisystem disorder of uncertain cause that is specific of human pregnancy. It is a newly onset hypertension in previously normotensive women coupled with or without elevated urine protein, developed after 20 weeks of gestation, as defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP)[9,10].

Hypertension is defined as a blood pressure of at least 140 mm Hg (systolic) or at least 90 mm Hg (diastolic) on at least two occasions and at least 4–6 h apart after the 20th week of gestation in women known to be normotensive beforehand[11]. Hypertension is regarded as severe if there are sustained rises in blood pressure to at least 160 mm Hg (systolic), at least 110 mm Hg (diastolic), or both[12]. Proteinuria is defined as excretion of 300 mg or more of protein every 24 h. If 24-h urine samples are not available, proteinuria is defined as a protein concentration of 300 mg/L or more (>1 + on dipstick) in at least two random urine samples taken at least 4–6 h apart[11].

Syndrome can be exacerbated to severe hypertension and proteinuria ("severe preeclampsia") that may lead to eclampsia, an obstetric emergency associated with brain convulsions, cerebral oedema and stroke, a life threatening condition for the mother and her baby[13]. HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome may correlate to severe preeclampsia and is characterized by elevated liver enzyme activities and reduced numbers of platelets indicating injury to the liver, blood system and potentially other organs. The HELLP syndrome occurs primarily in white, multiparous women above the age of 25 years[14].

According to the National Institute of Child Health and Human Development (NICHD) [15], confirmed by large scale studies of the Maternal-Fetal Medicine Foundation [16] and other international surveys [17], preeclamptic patients deliver prematurely (25% between 34-37 weeks of gestation and 10% before 34 weeks). The

more premature is the delivery, the more severe are the complications for both mother and child. The frequency of eclampsia, stroke, cerebral vascular accident and other severe complications is higher in preterm preeclampsia compared to term preeclampsia. More than 75% of babies born from early preeclampsia (<34 weeks) and over 50% of those born preterm (34–37 weeks) are either small for gestational age (SGA) or show intrauterine growth restriction (IUGR): these newborn account for the major fraction of intra-partum and post-partum death, cerebral palsy, blindness and motor and cognitive disorders. These complications are less severe in term preeclampsia[18 19 20].

We point out the two different aspects for this pathology: one involving the mother, and the other involving fetus. Pathogenesis and aetiology are still unclear.

Pathogenesis of pregnancy induced hypertensive disorders

It is well known that preeclampsia is associated with poor placentation and incomplete remodelling of the uteroplacental spiral arteries[21]. In this condition uterine spiral arteries fail to undergo the normal thinning out of muscular walls that permits enhanced perfusion of the placenta[22]. Thus, perfusion of the intervillous space is impaired, leading to placental hypoxia[23 24]. However the association is not specific. Poor placentation may occur with IUGR without features of preeclampsia[25] or with partial features such as pregnancy-induced hypertension. Poor placentation would be expected to cause IUGR. However IUGR is not a consistent feature of hypertensive disorders (HD) being confined largely to the early onset syndrome. In hypertensive disorders, at or beyond term, neonates are not growth restricted and may even be large for dates[26].

We consider a heterogeneous syndrome resulting by many factors.

To understand the pathophysiology of hypertensive disorders we have to consider the normal low grade systemic (vascular) inflammation response of the physiological pregnancy[27]. Pregnancy causes an intravascular inflammation that lead to endothelial

dysfunction: endothelial cells produce pro-inflammatory cytokines stimulating leukocytes, platelets and other humoral components[28]. Oxidative stress is both a cause and consequence of inflammation[29]. From the beginning of the pregnancy we note a maternal augmentation of pro-inflammatory index: leucocytosis[30], increasing IL-6 and TNF-α plasma concentration[31], fibrinogen[32], PAI-1[33]. Systemic inflammation induces also other metabolic adaptation to the pregnancy: in particular an insulin resistance and hyperlipidaemia develop in the second trimester[34 35].

This inflammation response is exacerbated in hypertensive diseases [35].

But it's important to consider many other predisposing factors to hypertensive disorders. Poor placentation is a separate disorder that typically leads to the maternal syndrome, depending on the degree to which it produces inflammatory signals. This inflammatory response may depend on fetal genotype and also on the maternal response to those signals, which depend on maternal genotype[36]. Many other pathophysiologic abnormalities have been suggested to explain mechanisms leading to the development of preeclampsia: immunologic intolerance between feto-placental and maternal tissues, placental and endothelial dysfunction, immune maladaptation to paternal antigens, exaggerated systemic inflammatory response, maladaptation to the cardiovascular or inflammatory changes of pregnancy, dietary deficiencies, and genetic abnormalities[37]. The opinion was that the clinical findings of hypertensive disorders could manifest as either a maternal syndrome (hypertension and proteinuria with or without other multisystem abnormalities) or fetal syndrome (fetal growth restriction, reduced amniotic fluid, and abnormal oxygenation)[38 39].

Many different classifications were used to describe hypertensive disorders [40 41]: mild, moderate, and severe, as well as early and late. The concept of early and late hypertensive diseases is more modern, and it is widely accepted that these two entities have different aetiologies and should be regarded as different forms of the probelm[41].

Early-onset form (before 34 weeks) is commonly associated with abnormal uterine artery Doppler, fetal growth restriction, evidence of ischemic lesions on placental examination and adverse maternal and neonatal outcomes[42 43]. In contrast, late-onset hypertensive disorder (after 34 weeks) is mostly associated with normal or slightly increased uterine resistance index, a low rate of fetal involvement, and more favourable perinatal outcomes[43 44]. Recently some Authors demonstrated that early-form and IUGR fetus could have the same origin, placenta mediated disease[45 46]: placental growth factor (PIGF), could be a useful second-trimester screening test for an early form of the disease, but not for late-onset preeclampsia/IUGR.

For this reason, two broad types of preeclampsia have been suggested: placental and maternal[43] preeclampsia.

It is generally agreed that poor placentation is strongly associated with IUGR, even in the absence of hypertension/preeclampsia, but it is less clearly documented in association with hypertension/preeclampsia and normal fetal growth. Thus, when Ness and Roberts pointed out that 70% of infants of preeclamptic women do not show IUGR, this suggested that abnormal placentation is not likely to be associated with the majority of cases of preeclampsia[43]. It later emerged that IUGR is a feature of early, not term, disease[47], suggesting that poor placentation is more likely to underlie this presentation.

Most hypertensive disorders occurs at term, that is, after 37 weeks' gestation[48]. Although term preeclampsia is less often associated with placental dysfunction, severe maternal complications can still occur. For example, 20% of cases with HELLP syndrome[49] and 55% of cases with eclampsia[49] occur at term. In other words, term hypertensive disorder is not benign just because the fetus is less threatened by IUGR, such as in early-onset preeclampsia.

Placental and maternal preeclampsia

Redman[50] wrote an article about the different kind of inflammation in preeclampsia and he suggested that the disease constitutes a spectrum that includes so-called 'maternal' and 'placental' preeclampsia.

This new concept retains there are two different pathogenesis of preeclampsia. The placental form is related to a placentation defect at the beginning of the pregnancy. The strongest hypothesis is that there is an inadequate placental invasion of the myometrium[51]. The throphoblast invasion is inhibited, the arteries are poorly remodeled and the capacity of the uteroplacental circulation is too small[52]. This condition leads to an oxidative stress with tissue damage and the release of placental factors, the first step for endothelial dysfunction and tissue inflammation[53]. All inflammatory networks are involved (leukocytes, platelet, endothelium, clotting cascade…) maybe enhanced by throphoblast debris into maternal circulation (syncytiotrophoblast microparticles, RNA-DNA of fetal origin, cytotrophoblast cells). Probably the increased release of trophoblastic debris is caused by apoptotic process and necrosis related to placental damage[54]

Maternal form is not necessarily associated with abnormal placentation and inadequate perfusion. There is a pre-existing endothelial and vascular dysfunction, in particular in women with risk factors: obesity, diabetes, metabolic syndrome are conditions that predispose the subject to develop the syndrome. Indeed adipose tissue is a very active endocrine organ[28]: Adipocytes produce and release numerous biologically active substances, such as leptin, adiponectin, interleukins (IL)-1, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), interferon-γ and monocyte chemo-attractant protein (MCP)-1, collectively termed adipokines[55]. This low-grade inflammation might explain the higher prevalence of preeclampsia in obese population. Pregnancy may constitute a metabolic and vascular stress test[52], which reveals a woman's health in later life and which is

consistent with the higher incidence of ischemic heart disease, stroke, and hypertension that becomes evident many years after an episode of preeclampsia[56].

In summary, we can consider placental preeclampsia as an abnormal placenta in a normal woman, and the maternal preeclampsia as a normal placenta within a woman who suffers from a pre-existing problem, such as obesity, metabolic syndrome or diabetes.

The pregnancy become a systemic inflammatory stress for women, particularly during the second half of gestation, to which both endocrine and placental factors contribute.

Placental preeclampsia is caused by an inadequate trophoblastic invasion of the maternal spiral arteries, that cause first a local inflammatory process and later a systemic inflammation, which has been documented by altered Doppler ultrasound of uterine arteries and often associated with a higher incidence of fetal growth restriction.

Conversely, maternal preeclampsia is associated with normal placentation and fetal growth and is thought to be a manifestation of an underlying metabolic disorder with chronic inflammatory state.

Further data support the hypothesis of two different causes leading to preeclampsia: pregnancy is characterized by certain structural and functional changes in the maternal cardiovascular systems. Adequate cardiovascular adaptation during early pregnancy leads to a state of high blood flow and low vascular resistance, which is a prerequisite to successful pregnancy outcome. In contrast, inadequate or excessive cardiovascular adaptation before 20 weeks of gestation is associated with pregnancies complicated by gestational hypertension, preeclampsia, IUGR or a combination of these[57].

In 2008, Valensise[58] observed two different groups of women that subsequently develop early or late preeclampsia. Patients with early onset preeclampsia had significantly higher rates of advanced maternal age, bilateral uterine arteries notching, lower gestational age at delivery and lower neonatal weight compared to both control and late

preeclampsia groups. In contrast, patients with late onset preeclampsia had higher body mass index compared to the other two groups.

Moreover he proposed that early and late onset preeclampsia develop from two distinct hemodynamic states: patients with early preeclampsia had significantly high total vascular resistance and lower cardiac output compared to those with late preeclampsia that present low vascular resistance and high cardiac output.

Valensise concludes that early preeclampsia appears to be more related to the evolution of an extremely altered cardiovascular response probably triggered by a placental disorder; instead, late preeclampsia seems to be more linked to maternal constitutional factors.

Prevention of preeclampsia: uterine artery Doppler velocimetry in first trimester

The primary prevention of any preeclampsia remains a considerable challenge in obstetrics. Although the symptoms of preeclampsia generally manifest themselves in the second to third trimester of pregnancy, their underlying pathology takes place in the first trimester[51].

Many researchers and physicians are still working to identify clinical elements in the first trimester to evaluate the effective risk of a woman to develop the disease, in order to allow targeted prenatal surveillance. Early prediction of preeclampsia would allow the development of treatments that could reduce the incidence of complications originating from this disease. Screening is valuable only if there is a therapeutic intervention that can improve the outcome. For this reason the efforts of the scientific community were concentrated on first trimester of pregnancy: indeed this is the best moment to modify the natural history of the pathology. This was definitely proved by usage of low-dose aspirin: in particular, it has been observed that the assumption of low-dose aspirin after the 16^{th} week in patients with risk factors slightly reduces the

incidence of early preeclampsia[59]. When used before the 16th week, the relative risk for preeclampsia is sensibly reduced[60].

The most recent meta-analysis that has been published further demonstrates that low-dose aspirin started before16 weeks' gestation was particularly effective in preventing preterm preeclampsia rather than term preeclampsia (RR: 0.11, 95% CI: 0.04–0.33 versus RR: 0.98, 95% CI: 0.42–2.33)[61]. Successful implementation of this intervention is therefore dependent on being able to screen effectively in early pregnancy. For this reason current clinical risk assessment for preeclampsia is done in the first trimester[62] for early identification of women who can benefit of preventative treatment such aspirin[63]. This includes women with at least one high risk factor (previous history of hypertension in pregnancy, chronic kidney disease, autoimmune disease, type 1 and 2 diabetes and chronic hypertension) or two moderate risk factors (first pregnancy, aged 40 years or more, pregnancy interval of more than 10 years, Body Mass Index BMI of 35 or more, family history of preeclampsia, multiple pregnancy).

The traditional approach was based on maternal history and systematic use of systemic mean arterial pressure (MAP)[64]. Thus, for women considered at high risk for preeclampsia, due to previous preeclampsia or chronic hypertension or diabetes or multi-fetal pregnancy, the frequency of those who will develop preeclampsia is 19% (previous preeclampsia), 22% (diabetes) and 25% (chronic hypertension)[65]. Although the screening by maternal history alone will detect only 30% of women who will develop preeclampsia, the clinical risk-based strategy is not effective for nulliparous women without other risk factors[66].

Many other biophysical and biochemical parameters were introduced for the early identification of preeclampsia. In particular uterine artery Doppler pulsatility index (UtA-PI) was studied during second trimester[67], and serum placental markers[68]. These methods alone do not achieve detection rates sufficient to be used as screening test.

A great innovation in obstetrics was brought by the introduction of the uterine arteries Doppler velocimetry in clinical practice. Uterine arteries analysis gives useful information on placentation: this non-invasive exam provides information on the otherwise unknown placental tissue, the maternal-placental vascularization state, and further additional deviations from physiology towards pathology. The evaluation of uterine arteries in the first trimester may unveil the secrets of early placental development. Many studies were based on the predictive value of uterine arteries for preeclampsia in the first trimester[69 70]. There is a stronger association between UtA-PI and early-preeclampsia, with respect to late-onset preeclampsia, probably for the different aetiology of the disease. Indeed we could consider uterine arteries as a mirror of placental function and development.

A recent meta-analysis[71] of 55,974 women, observed that the predictive capacity of uterine arteries to detect early-preeclampsia in a low risk population has the same performance as clinical high risk factors. This makes a strong case for introducing uterine artery Doppler assessment in the first trimester and to develop a prediction model incorporating the clinical characteristics with uterine artery Doppler to increase the accuracy of risk assessment[64].

Since fetal growth is a multifactorial process in which placentation is only one of the factors involved, the use of a single parameter such as Doppler velocimetry remote from the delivery to predict birth weight in a low-risk population seems to be useless[72]. Fetal growth restriction is caused by multiple aetiologies including aneuploidy, genetic syndromes, environmental factors, drug abuse, smoke, intrauterine infections, maternal thrombophilia. The prediction of the pathophysiologic process through the uterine arteries in the first trimester seems to be poor. Uterine arteries predict better early IUGR, because of its strong association with preeclampsia[73].

In order to improve the prediction of the disease, many authors have combined the patient history with a series of biophysical and biochemical markers that change from as early as the first trimester of pregnancy in cases that subsequently develop preeclampsia or IUGR. Studied biophysical markers include mean arterial blood pressure[74], uterine artery Doppler[75] and more complex evaluations such as maternal cardiac output[76] and brain hemodynamic measurements[77]. Several biochemical markers have been tested for the prediction of preeclampsia and IUGR, including products of fetal and placental origin, markers of renal or endothelial damage, angiogenic and antiangiogenic factors, and markers of oxidative stress as reviewed by Giguère in 2011[78]. Maternal serum markers, such as inhibin A, activin A, soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin, when used alone are proved poor predictors of preeclampsia[79 80].

Aim of the study

Pregnancy induced hypertension is a complex and heterogeneous spectrum of pathologies that involves 5-10% of pregnancies. It is one of the major contributors to maternal and perinatal mortality and morbidity worldwide.

Until now the classification of these diseases was based on timing of insurgence or delivery, or clinical manifestation using the grade of the pathology.

The aetiology of this syndrome was not considered.

In the last years, many Authors focused their attention on the heterogeneity of this group of disease, underline the different origin between the placental and maternal preeclampsia. The first one is associated with fetal growth restriction, alteration of placenta and fetal Doppler velocimetry and important maternal involvement, thought to be a consequence of impaired placentation. This condition leads to an oxidative stress with tissue damage and the release of placental factors, the first step for endothelial

dysfunction and tissue inflammation with possible consequence on fetal growth. The second one is associated with normal fetus-placental blood flow and rarely with important clinical involvement for the mother. The underlying pathophysiological process to this conditions thought to be the metabolic syndrome with chronic low-grade inflammation and increased insulin resistance characterizing typically women with android obesity. This is the rationale to support the theory that stress mainly the aetiology of the disease, and that considers preeclampsia of placental origin associated with IUGR, while preeclampsia of maternal origin associated with appropriately growth fetus (AGA).

Despite improvement in the understanding of the pathophysiology of these conditions, ability to accurately predict pregnant woman who will develop preeclampsia is limited. This greatly impairs the development and testing of preventive interventions.

All screening tests using to early detection of women at high risk of this pathology considered the classification of hypertensive disease in early-onset HD and late-onset HD. This is a rigid and strained classification that not considered the various possibility of a complex syndrome independently from gestational age.

We want to consider the aetiology of the pathologies, through an accurate definition and study of symptoms and signs of the disease.

The aim of the study is to evaluate a new classification of hypertensive disease based on physiopathology, and not on temporary factors. We want to study uterine arteries diagnostic power on first trimester to predict hypertensive disorders.

Material and Methods.

This is a prospective longitudinal cohort study of pregnant women followed in two hospital: Prenatal Diagnosis and Gynaecologic Unit of the Institute for Maternal and Child Health – IRCCS "Burlo Garofolo" in Trieste and Obstetrics and Gynaecologic Unit of Children's Hospital – ICP "Vittore Buzzi" in Milan, Italy.

The study was approved by Ethics Committee of the Institute for Maternal and Chid Health – IRCCS Burlo Garofolo and of the Istituti Clinici di Perfezionamento (ICP)-Buzzi Children's Hospital.

This study was offered to pregnant women at the time of first trimester ultrasound aneuploidy screening. All women were recruited consecutively from October 2007 to April 2009 in Triest and from October 2009 to December 2012 in Milan.

We enrolled singleton pregnancies between 11+0 and 13+6 weeks of gestation.

All pregnancies were dates by last menstrual period if consistent with crown-rump length measurements (± 7 days), or by CRL measurements if it was not consistent with menstrual dating.

The inclusion criteria were:

- single pregnancy;
- absence of fetal anomalies;
- age major to 18;
- obtained informed consent;

The exclusion criteria were:

- multiple pregnancy;
- presence of fetal anomalies (fetal malformation, aneuploidy, congenital syndrome);
- miscarriage before 20 weeks;
- termination of pregnancy (TOP);

- intrauterine fetal-death;
- age inferior to 18;
- informed consent not obtained.

During the first visit a written informed consent was collected. At the time of inclusion, women had an interview with a researcher and answered a standardized questionnaire on maternal characteristics and medical history. Demographic and clinical data included: age, racial origin (Caucasian, African, Asian, East Asian and Mixed), smoking habit, family history of hypertension, medical pathologies, drug assumption, parity (nulliparous if no delivery beyond 20 weeks), method of conception (spontaneous or IVF), obstetrical history.

The maternal weight and height were measured and the body mass index (BMI) was calculated.

We performed ultrasound examinations measuring fetal CRL, Nuchal Translucency (NT) and Nasal Bone (NB). During the ultrasound exam, Doppler velocimetry of uterine arteries (left and right) was performed. All indices were recorded: Pulsatility Index (PI) and Resistant Index (RI). For the purposes of this study the mean value of the pulsatility index from the left and right uterine artery was used (Mean UtA PI)[81]. Maternal serum PAPP-A and free β Human Chorionic Gonadotropin (β-hCG) were determined to calculate the combined patient-specific risk for trisomy 18 and 21[82 83].

All participating sonographers are certified by the Fetal Medicine Foundation for first trimester screening. The ultrasound scan was performed using a high-resolution, real-time ultrasound equipment (Voluson E8 and Voluson 730 Expert, General Electric Medical Systems, Milwaukee, WI, USA) equipped with a 5-MHz to 7.5-MHz curvilinear transabdominal probe.

Uterine arteries Doppler velocimetry assessment

Both uterine arteries were examined as suggested by the Fetal Medicine Foundation, London, UK (www.fetalmedicine.com/fmf) by an operator certified by the Fetal Medicine Foundation for the first trimester screening. A sagittal section of the uterus was obtained and the cervical canal and internal cervical os identified. The transducer was tilted from side to side and color flow mapping was used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os. Pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel and with care taken to ensure that the angle of insonation was less than 30°. When three similar consecutive waveforms were obtained, the UtA-PI and UtA-RI were measured and the mean UtA-PI was determined. The first trimester Doppler results were not shown in the ultrasound report but the mean PI was used for analysis. The presence or absence of an early diastolic notch was not included in the analysis[84].

Laboratory technique

Once the ultrasound examination was performed and the gestational age confirmed, a blood sample (5 cc) was taken from each woman.

Maternal serum PAPP-A was measured using a kit for B.R.A.H.M.S KRYPTOR automated immunofluorescent assays (Hennigsdorf, Germany; www.kryptor.net) in Milan and DELFIA Xpress (Perkin Elmer Life and Analytical Sciences, Turku, Finland) in Triest. PAPP-A and β-hCG values were converted in multiple of the median (MoM), which were corrected for CRL and body mass index (BMI). The samples were analysed by an examiner blinded to the clinical outcomes.

Outcome data collection

Pregnancy outcome data were collected as follows: fetal and maternal outcomes were obtained either directly from the clinical record if the delivery occurred in "Vittore Buzzi" Hospital or in "Burlo Garofolo" hospital or by a telephone questionnaires to the women after delivery.

Definition of outcomes

Adverse pregnancy outcome was defined as the presence of one or more of the following:

- Pregnancy induced hypertension (PIH):

  - *Gestational hypertension (GH)* was defined as diastolic blood pressure above 90 mm Hg or more on at least two occasions, at 4 hours apart, developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria[85].

  - *Preeclampsia (PE)* was defined as diastolic BP of at least 110 mmHg on one occasion or diastolic BP of at least 90 mmHg on two consecutive occasions more than four hours apart, in combination with proteinuria (≥300 mg total protein in a 24-hour urine collection or, if this was not available, ≥ +2 proteinuria by dipstick analysis on two consecutive occasions at least four hours apart) developing after 20 weeks of gestation in previously normotensive women[85].

  For the purpose of the analysis we decided to introduce a new classification of hypertensive disorders (HD) according with recent literature: HD of placental or maternal origin.

  Placental-hypertensive disorder (placental-HD) is characterized by PIH and impaired fetal growth, defined as fetus with ultrasound abdominal circumference below the10th

centile or a drop in abdominal circumference > 40th centiles in two consecutive ultrasound examinations.

Maternal-hypertensive disorder (maternal-HD) is characterized by PIH with a fetus appropriate for gestational age, independently of gestational age at diagnosis.

In a second time we considered also standard classification of HD based on time of delivery:

- Early onset-HD (PIH and age at delivery before 34 week of gestation).
- Late onset-HD (PIH and age at delivery after 34 week of gestation)[86].

- Chronic hypertension (CH) was defined as diastolic blood pressure above 90 mm Hg or more on at least two occasions, at 4 hours apart, developing before pregnancy or before 20 weeks of gestation[85].

- Intra-uterine growth restriction (IUGR) was defined as ultrasound abdominal circumference below the 10th percentile according to standards references based on gestational age. Other causes of IUGR such as infection, anomalies and abnormal chromosomes were excluded in all cases[87].

In a preliminary study we considered as outcome also:

- Gestational diabetes: positive 75 gr oral glucose tolerance test during pregnancy
- Spontaneous preterm labour: delivery of a live baby before 37 weeks of gestation.
- Large for gestational age fetuses (LGA): a birth weight over the 90th percentile according to standards references for weight based on gestational age[87].

Statistical analysis

We started with a descriptive analysis of gestational outcome, separating the unaffected group from the groups of women affected by PHI, CH, SGA fetus, LGA fetus, GDM, PTL. In a preliminary analysis we considered women with preterm labour, diabetes and fetus LGA, but we noted that there were no difference from control group.

So we decided to consider women with preterm labour, gestational diabetes and LGA fetuses in the control group creating 4 case groups to the definitive analysis.

We used two different classifications for the outcome. The first one considered hypertensive disorders in placental and maternal. The second one considered hypertensive disorders on time of delivery (early and late).

We compared demographic characteristics (maternal age, BMI, ethnicity, parity, conception, gestational age at delivery, infant birth-weight), uterine mean PI values, PAPP-A and β-hCG levels among groups, for every classification.

Data were expressed as mean ± 95% CI for continuous variables and as number and percentage for categorical variables. The distribution of data was evaluated with Kruskall-Wallis test. The results are represented as mean value and standard deviation (SD). Logarithmic transformation was performed to normalize mean UtA-PI. Logistic regression models were computed to evaluate the significance of the variables considered. The following variables were included: UtA mean PI, BMI, gestational age at time of recruitment and maternal age. The receiver operating curves (ROC) constructed on regression models were computed and area under the ROC (AUC) calculated to evaluate the performance of the model. We evaluated the 1st trimester model to identify: firstly, women at risk of developing placental or maternogenic HD, and, secondly, the early and late-onset HD. Each disease was evaluated against the whole cohort. The area under the curves was calculated and compared with the roctab and roccomp function in STATA. The test characteristics at 5% and 10% fixed false

positive rate were calculated with the roctab function and the estat classification function at the cut-off value at the 95th and 90th percentile for negative outcome.

The analysis was performed with the program Stata/IC 11.2 for Windows (Stata Corp LP, College Station, USA).

Results

4218 pregnant women were recruited in occasion of their ultrasound first trimester screening. At the moment of the analysis 712 patients were lost to follow up or excluded because incomplete data acquisition. This resulted in the cohort of 3506 women with complete outcome follow-up, of which 18 women had first/second trimester miscarriage, 10 women performed a termination of pregnancy and 6 women had intrauterine fetal death. These patients were excluded from analysis.

Among 3472 pregnancies included in the study, 122 women (3,5%) developed some hypertensive gestational disease, 56 fetuses were IUGR (1,6%), 10 women had chronic hypertension (0.3%) and 3284 women were unaffected (94.6%).

If we considered PIH classification based on the aetiology, 16 women (0.5%) developed placental HD, 106 (3.0%) presented maternal HD.

Table 1 reports maternal and fetal outcomes occurred in the study groups using classification of placental- and maternal- HD.

OUTCOME	**3472**
PLACENTAL HD (GH/PE + IUGR)	*16 (0.5%)*
MATERNAL HD (GH/PE + AGA)	*106 (3.0%)*
CH	*10 (0.3%)*
IUGR	*56 (1.6%)*
UNEFFECTED	*3284 (94.6%)*

Table 1

If we considered classification based on time of delivery, 11 women (0.3%) developed early-HD, 111 women (3.2%) presented late-HD.

In Table 2 we report the same data using classical classification of HD: early- and late-onset HD.

OUTCOME	**3472**
EARLY HD (< 34 WS)	*11 (0.3%)*
LATE HD (> 34 WS)	*111 (3.2%)*
CH	*10 (0.3%)*
IUGR	*56 (1.6%)*
UNEFFECTED	*3284 (94.6%)*

Table 2

	PLACENTAL HD (GH/PE + IUGR)	***MATERNAL HD (GH/PE + AGA)***	
EARLY HD (< 34 WS)	9	2	11
LATE HD (> 34 WS)	7	104	111
	16	106	

Table 3

Demographic variables into groups

Table 4 shows the main features of case group, placental HD, maternal HD, CH group and SGA group.

Variable	Unaffected (n=3284)	Plac HD (n=16)	Mat HD (n=106)	CH (n=10)	IUGR (n=56)
Age, years, mean (95%CI)	32.6 (32.4-32.7)	31.8 (29.2-34.4)	33.2 (32.4-34.0)	34.8 (31.3-38.4)	31.7 (30.5-33.0)
BMI, Kg/m^2, mean (95%CI)	22.3 (22.2-22.4)	24.2* (22.1-26.2)	24.7** (23.8-26.2)	30.4** (26.1-34.8)	21.6 (20.8-22.5)
Ethnicity: Caucasian, %	96.0	93.8	95.3	100.0	94.6
Parity : nulliparous, %	60.6	71.4	69.6	70.0	63.6
Conception: spontaneous, %	97.0	100.0	91.9	80.0	100.0
Gestational age at delivery, weeks (95%CI)	39.5 (39.5-39.6)	33.0** (31.0-35.0)	38.7** (38.3-39.1)	39.6 (38.7-40.5)	38.7** (38.2-39.1)
Infant birth weight, gr, mean (95%CI)	3351 (3335-3367)	1589** (1262-1916)	3104** (2986-3223)	3359 (3094-3623)	2487** (2397-2576)

* for p<0.05; ** for p<0.01: comparison with group of unaffected with Mann-Whitney rank-sum test or exact Fisher two-tailed test, as appropriate.
Plac = placental; HD = hypertensive disease; Mat = maternal; CH = chronic hypertension; IUGR = intrauterine growth restriction; gr = grams; 95%CI = 95% confidence interval.

Table 4

There were no statistically significant differences in age, ethnicity, parity and conception among every case and control group. There were difference in BMI, gestational age at delivery and infant birth-weight. Effectively, BMI at the enrolment was significant higher in women with CH and maternal HD (p<0.01), placental HD (p<0.05).

There were a significant lower gestational age at delivery and a lower birth-weight in women with placental and maternal HD, IUGR fetuses (p<0.01).

Variable	Unaffected (n=3284)	Early-HD (n=11)	Late-HD (n=111)	CH (n=10)	IUGR (n=56)
Age, y, mean (95%CI)	32.6 (32.4-32.7)	33.5 (31.4-35.6)	32.9 (32.1-33.8)	34.8 (31.3-38.4)	31.7 (30.5-33.0)
BMI, Kg/m^2, mean (95%CI)	22.3 (22.2-22.4)	24.7 (22.3-27.0)	24.6 (23.7-25.5)	30.4** (26.1-34.8)	21.6 (20.8-22.5)
Ethnicity: Caucasian, %	96.0	100.0	94.6	100.0	94.6
Nulliparous, %	60.6	77.8	69.2	70.0	63.6
Conception spontaneous, %	97.0	100.0	92.3	80.0	100.0
Gestational age at delivery, weeks (95%CI)	39.5 (39.5-39.6)	30.0** (28.4-31.6)	38.7** (38.4-39.1)	39.6 (38.7-40.5)	38.7** (38.2-39.1)
Infant birth weight, gr, mean (95%CI)	3351 (3335-3367)	1158** (864-1452)	3079** (2967-3191)	3359 (3094-3623)	2487** (2397-2576)

* for p<0.05; ** for p<0.01: comparison with group of unaffected with Mann-Whitney rank-sum test or exact Fisher two-tailed test, as appropriate.
Plac = placental; HD = hypertensive disease; Mat = maternal; CH = chronic hypertension; IUGR = intrauterine growth restriction; gr = grams; 95%CI = 95% confidence interval.

Table 5

Using the second classification with standard definition of HD (early- and late-) BMI at the enrolment was significant higher only in women with CH (p<0.01). There was a significant lower gestational age at delivery and a lower birth-weight in women with early and late HD and IUGR fetuses (p<0.01) (table 5).

Placental parameters: Doppler velocimetry of the uterine arteries and biochemical markers in placental and maternal hypertensive disease.

The results of Doppler examination of the uterine arteries and serum markers analysis are represented in Table 6.

Variable	Unaffected (n=3284)	Plac HD (n=16)	Mat HD (n=106)	CH (n=10)	IUGR (n=56)
Mean UtA PI Log10 (95%CI)	1.60 (1.58-1.61)	2.36** (2.10-2.62)	1.68 (1.57-1.78)	1.54 (1.18-1.89)	1.79** (1.64-1.94)
PAPP-A MoM (95%CI)	1.08 (1.06-1.11)	0.88 (0.67-1.09)	0.93** (0.79-1.08)	0.71* (0.55-0.87)	0.92** (0.70-1.13)
BhCGMoM (95%CI)	1.13 (1.11-1.16)	1.15 (0.73-1.57)	1.13 (0.96-1.30)	1.06 (0.52-1.61)	0.95 (0.80-1.10)

* for p<0.05; ** for p<0.01: comparison with group of unaffected with Mann-Whitney rank-sum test.

Table 6

The mean uterine artery PI was significantly higher in placental HD and in IUGR group (p<0.01) when compared with the unaffected group (table 6, figure 1). PAPP-A was significant lower in maternal HD and IUGR group (p< 0.01) and in CH group (p<0.05). There was no significant difference in B-hCG levels through study group.

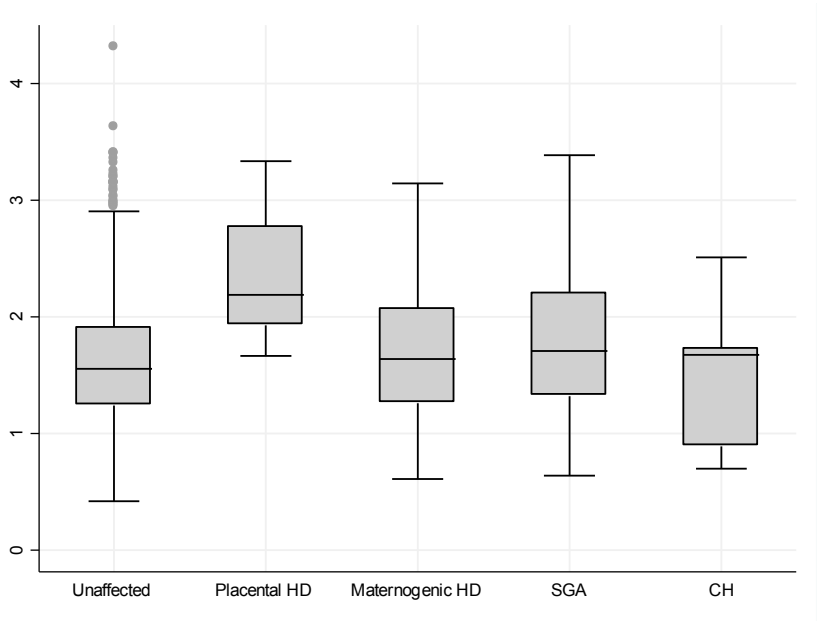

Figure 1

Placental parameters: Doppler velocimetry of the uterine arteries and biochemical markers in early and late hypertensive disease.

If we consider the early-late group, the mean uterine artery PI was significantly higher in early HD group and IUGR group ($p< 0.01$) respect control group and in late HD group ($p< 0.05$) (Table 7, figure 2). PAPP-A was significant lower in late-HD and IUGR group ($p< 0.01$) and in CH group ($p<0.05$). No significant differences between groups for B-hCG.

Variable	Unaffected (n=3284)	Early-HD (n=11)	Late-HD (n=111)	CH (n=10)	IUGR (n=56)
Mean UtA PI Log10 (95%CI)	1.60 (1.58-1.61)	2.30** (2.00-2.60)	1.71* (1.61-1.82)	1.54 (1.18-1.89)	1.79** (1.64-1.94)
PAPP-A MoM (95%CI)	1.08 (1.06-1.11)	0.79 (0.62-0.96)	0.94** (0.80-1.08)	0.71* (0.55-0.87)	0.92** (0.70-1.13)
BhCG MoM (95%CI)	1.13 (1.11-1.16)	1.00 (0.75-1.26)	1.15 (0.98-1.32)	1.06 (0.52-1.61)	0.95 (0.80-1.10)

* for $p<0.05$; ** for $p<0.01$: comparison with group of unaffected with Mann-Whitney rank-sum test.

Table 7

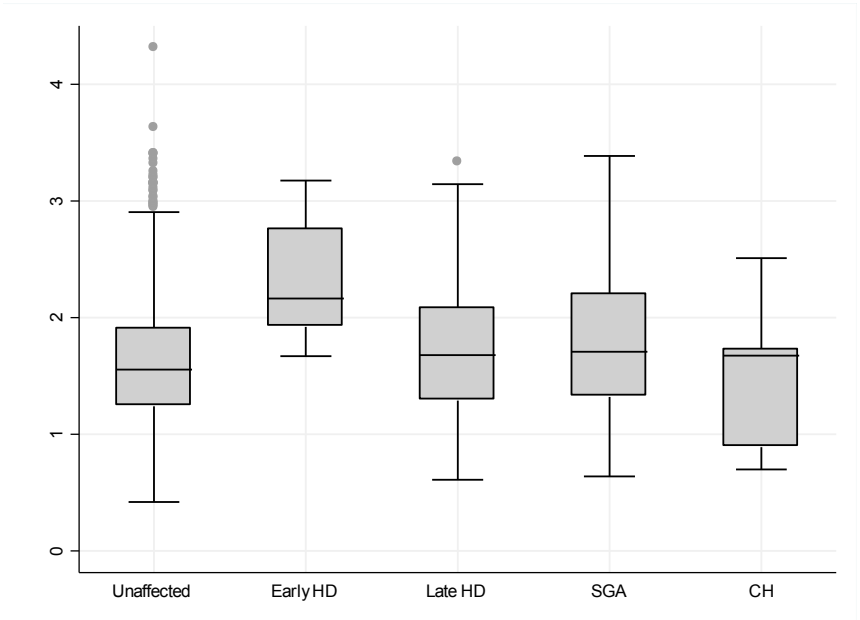

Figure 2

Placental-maternal model: comparing each group with the other.

The results of regression logistic analysis of every group of placental-maternal model with the other, are represented in table 8.

	Placental HD		Maternal HD	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
PI uterine arteries mean	11.7 (4.56-30)	< 0.001	1.26 (0.85-1.86)	0.24
Age	0.94 (0.84-1.05)	0.26	1.02 (0.98-1.07)	0.3
BMI	1.12 (1.01-1.26)	0.03	1.13 (1.09-1.18)	< 0.001
Gestational age	1.04 (0.37-2.95)	0.93	0.90 (0.61-1.32)	0.61

Table 8

We calculated the area under the ROC curve (AUC) for placental and maternal HD. Comparing placental HD with all other groups, area under the curve was 0.879 (Figure 3).

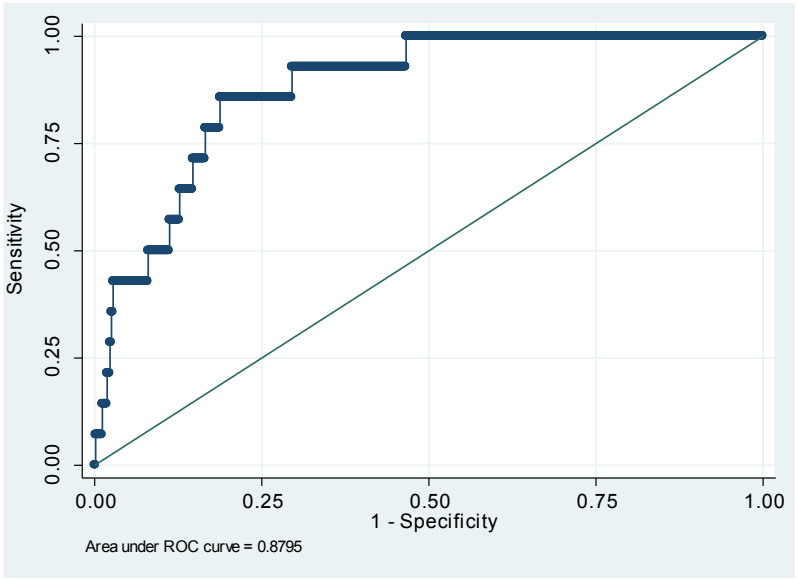

Figure 3

Comparing maternal HD with all other group, area under the curve was 0.666 (figure 4).

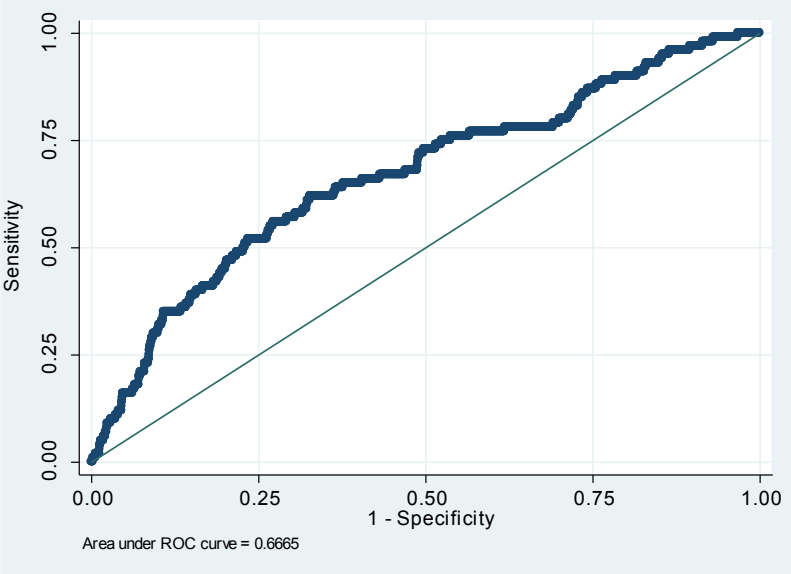

Figure 4

Early and late model: comparing each group with the other.

The results of regression logistic analysis of every group of early-late model with the other, are represented in table 9.

	early HD		late HD	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
PI uterine arteries mean	7.64 (2.49-23.40)	< 0.001	1.49 (1.02-2.16)	0.04
Age	1.02 (0.89-1.17)	0.76	1.01 (0.96-1.05)	0.61
BMI	1.14 (1.00-1.30)	0.04	1.13 (1.09-1.17)	<0.001
Gestational age	0.56 (0.14-2.17)	0.40	0.95 (0.65-1.39)	0.81

Table 9

Comparing early HD with all other groups, area under the curve for was 0.858 (figure 5).

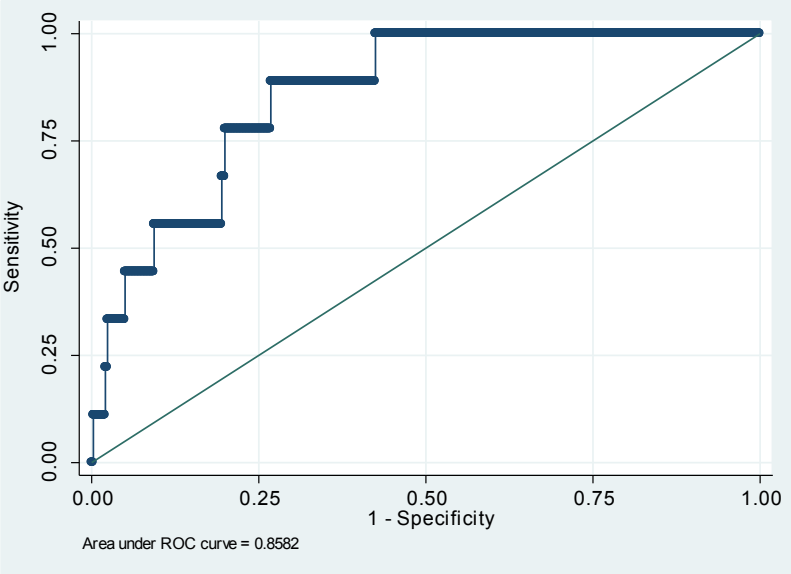

Figure 5

Comparing late HD with all other groups, area under the curve for was 0.668 (figure 6).

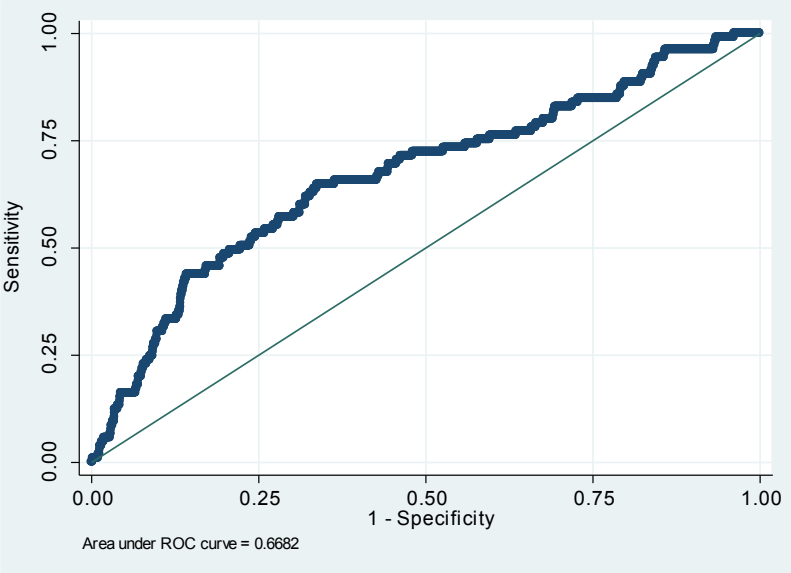

Figure 6

Table 10 shows the performance of first trimester screening with the two classifications (5% and 10% false positive rate).

	5% false positive			10% false positive		
	sensitivity	**LR+**	**LR-**	**sensitivity**	**LR +**	**LR -**
Placental HD	44%	10.65	0.59	50%	5.55	0.55
Maternal HD	16%	3.20	0.88	28%	3.10	0.79
Early HD	33%	8.23	0.69	44%	4,76	0.61
Late HD	13%	3.20	0.90	25%	2.59	0.81

Table 10

Placental versus Maternal hypertensive disorders

Comparing placental HD with maternal HD, mean uterine arteries PI was significantly higher in hypertensive disorders of placental origin with OR of 12.84 (p = <0.001, Table 11).

	OR (95% CI)	**P**
PI uterine arteries mean	12.84 (3.27-50.38)	< 0.001
Age	0.89 (0.77-1.04)	0.14
BMI	1.04 (0.90-1.23)	0.54
Gestational age	1.35 (0.38-4.80)	0.64

Table 11

Comparing placental with maternal HD, area under the curve was 0.844 (figure 7).

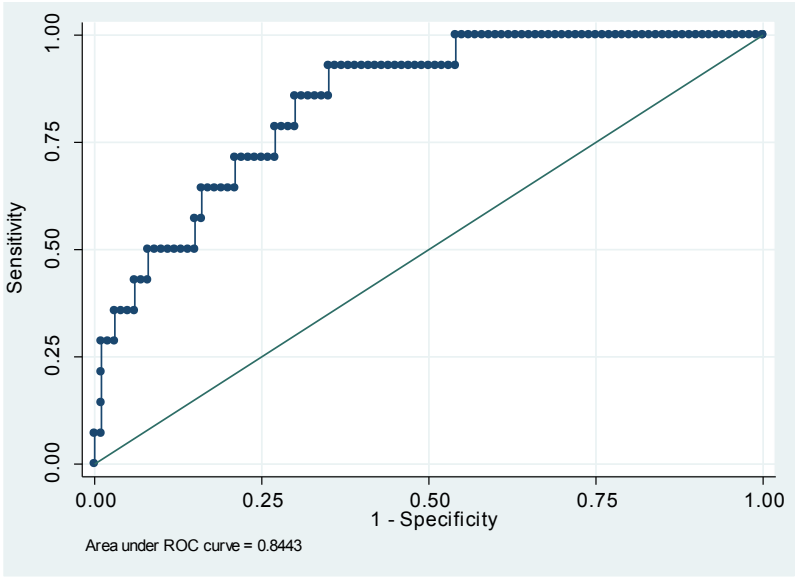

Figure 7

Comparing early and late HD, there was not the same result (table 12).

	OR (95% CI)	P
PI uterine arteries mean	5.50 (1.44-20.91)	0.01
Age	1.03 (0.86-1.22)	0.77
BMI	1.07 (0.90-1.29)	0.48
Gestational age	0.62 (0.14-2.64)	0.51

Table 12

The area under the curve (AUC) was 0.775 (figure 8).

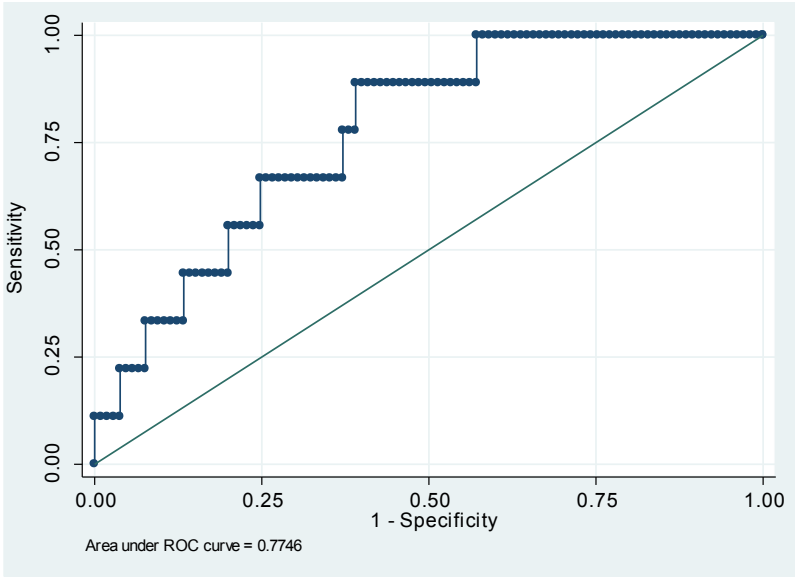

Figure 8

IUGR and CH: comparing each group with all the population.

The results of regression logistic analysis of IUGR and CH group with the other population are represented in table 13.

	IUGR		CH	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
PI uterine arteries mean	1.95 (1.18-3.25)	0.01	1.04 (0.30-3.57)	0.95
Age	0.96 (0.90-1.01)	0.14	1.11 (0.96-1.29)	0.16
BMI	0.94 (0.86-1.01)	0.16	1.30 (1.18-1.43)	<0.001
Gestational age	0.84 (0.49-1.44)	0.53	0.67 (0.20-2.29)	0.52

Table 13

Comparing IUGR with all other groups, area under the curve for was 0.626 (figure 9).

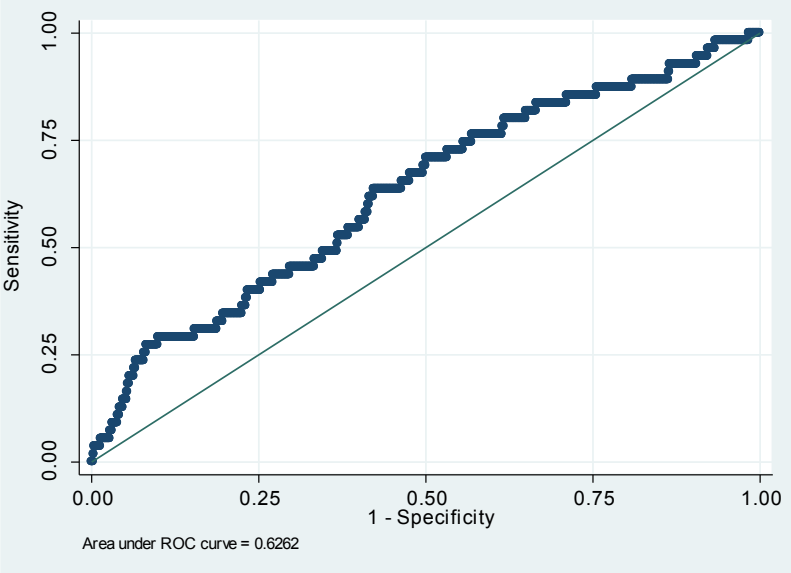

Figure 9

Comparing CH with all other groups, area under the curve for was 0.833 (figure 10).

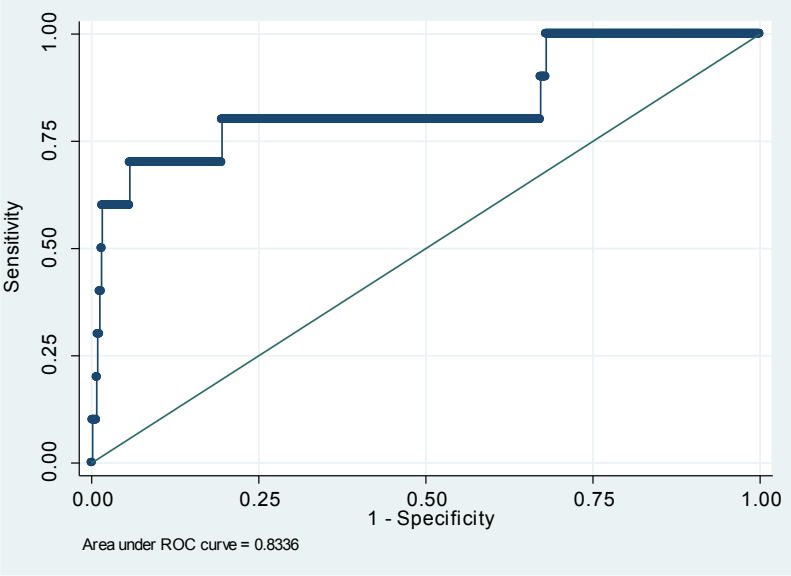
Sensitivity
0.00
0.25
0.50
0.75
1.00
0.00
0.25
0.50
0.75
1.00
1 - Specificity
Area under ROC curve = 0.8336

Figure 10

Discussion

The aim of our study is to validate a new classification of gestational hypertensive disorders recently proposed in the scientific community.

Gestational hypertensive disorders is commonly classified according to gravity (mild, moderate, severe) or according to gestational age at insurgence (or at delivery). Nevertheless, the clinical and physiopathological features of the disease are not included in this type of classification. It is commonly believed that there exist two types of hypertensive disorders, originating by different physiopathological conditions. Disorders of placental origin, where gestational hypertension or preeclampsia are associated to IUGR, and maternal hypertension diseases, where the fetal growth appears within normal ranges, independently of gestational age at the diagnosis. Placental and maternal hypertensive diseases recognize different aetiologies and, therefore, develop through different models of maternal cardiovascular adaptation in the latent phase of the disease.

Scientific papers in recent years have recognized in the early-late classification a different physiopathological expression of the disease, assigning the early one to a placental defect, and thus leading more often to problems concerning the fetal growth. Late preeclampsia, instead, is more correlated to maternal problems. We think that a classification only based on time is not enough: placental preeclampsia appears quite often at an early stage, but it is not unusual at later times with IUGR fetuses as well. In our series 9 placental diseases developed before 34 weeks and 7 after 34 weeks.

The analysis of uterine arteries in first trimester has been used in many studies as a predictive marker for placental pathologies: this kind of analysis was previously limited to second and third trimester only. Since the placental development is not completed at an early stage (first trimester), it is very important to identify risk factors at this age that

could lead to a placental pathology, in order to plan a series of preventive strategies (drugs, diet, behaviour).

Accurate prediction of gestational hypertensive disorders and intrauterine growth restriction is crucial to allow judicious allocation of resources for monitoring and the development of preventive treatment to improve maternal and perinatal outcomes. Early identification of women at risk of gestational hypertension diseases facilitates targeted surveillance and intervention[88]. There are likely to be significant advantages in predicting hypertensive diseases during the first trimester, as opposed to the second; since the disease process is already established by the mid second trimester, any successful preventative measure will need to be instituted in pregnancy as early as possible.

Uterine arteries

In our study, we investigated uterine arteries during the first trimester in healthy women that have later developed hypertensive disease. We choose to analyse the interval between the 11th and 13th week of gestation because in this period women use to make the combined sonographic and biochemical testing for chromosomal and other major defects. We used the two classifications of hypertensive disorders: the time-based definition (early: before 34 weeks, late: after 34 weeks) and the physiopathological-based (placental is associated to IUGR, and maternal when the fetal growth appears within normal ranges).

In our work IUGR assessment was based on biometric prenatal evaluation and not on the birth weight of the fetus. This led us to consider only those small fetuses recognized during pregnancy, and not considering as IUGR fetuses small at birth and not diagnosed prenatally.

We observe that a different choice of criteria affect the predictive power of uterine arteries. Mean uterine arteries PI was significantly higher, both in case of placental and early hypertensive diseases, while no differences were observed between maternal and late hypertensive diseases compared to controls. The difference was even more evident using the new etiologic classification.

Concerning prediction of the logistic regression, the validity of the uterine arteries Doppler velocimetry has been confirmed for early identification of women at risk of developing a hypertensive disease, especially of placental origin. In effect the area under the ROC curve for placental hypertensive diseases was 0.879, whereas for early diseases was 0.858.

Instead, uterine artery Doppler velocimetry in maternal and late hypertensive diseases was comparable to that of the general population. These data confirm the hypothesis of existence of two different hypertensive disorders characterized by different aetiology and clinic, but not recognized only by temporal criteria. In fact, Doppler-velocimetry study was more effective in identification of hypertensive disorders especially of placental origin, as well as the early ones.

The literature suggests that early preeclampsia/IUGR is associated with defective invasion of the spiral arteries, whereas the spiral artery defect plays a much smaller role in the cases closer to term[89]. Thus, term preeclampsia/IUGR seems to be associated with normal trophoblast transformation in the first trimester, and late atherosclerotic changes in spiral arterioles. Such late changes may be the consequence of increased placental mass, as occurs in diabetic and twin pregnancies, related to the senescence of the placenta in prolonged pregnancy or as a result of placental edema and necrosis in fetal hydrops[90].

Abnormal uterine artery Doppler studies in the first trimester have been shown to be associated with preeclampsia/IUGR. Gomez et al. reported that the sequence of changes

in uterine artery Doppler between the first (11 and 14 weeks) and second (20 and 24 weeks) trimesters correlates with the subsequent development of preeclampsia and IUGR[91].

The detection rate of uterine artery screening for preeclampsia at any gestation is better for severe than for mild disease. Increased resistance indices in the first trimester are particularly effective in identifying preterm, rather than term, preeclampsia[92 93 94].

In addition, uterine artery Doppler in the first and the second trimesters was shown to have a remarkably higher sensitivity in women with preeclampsia complicated by small-for-gestational-age (SGA) babies compared with uncomplicated preeclampsia or SGA alone[95].

Our study are in agreement with the literature data and shows that the mean uterine artery PI was significantly higher in women who subsequently developed placental hypertensive diseases, but not in maternal hypertensive disorders, confirming indirectly our hypothesis that maternal form is not associated with impaired placentation.

In the last 10-15 years hypertensive disorders classification in early and late, based at the time of onset before or after 34 weeks of gestation, was the most commonly used classification. The classification based exclusively on a temporal criteria has not been sufficient to assess these diseases. Nevertheless, all screening tests developed until now based on this temporal classification: maybe this is why that they proved inadequate.

Moreover in our study the physiopathological-based classification allowed us to identify 5 additional cases respect to the early-late classification (placental HD n= 16. Early HD n=11). This is very important for clinical application. Placental form represents a disease able to compromise the pregnancy on various fronts (maternal and fetal) making the picture much more complex.

Uterine arteries Doppler in first trimester didn't show significant correlation with IUGR e CH group according to the literature: for IUGR, the sensitivity of uterine arteries Doppler in the first trimester was 15%[71].

PAPP-A

PAPP-A is an insulin-like growth factor binding protein (IGFBP) protease with specificity for IGFBP 2 and 4. Reduced levels of PAPP-A may result in increased amounts of insulin-like growth factor (IGF) being bound to its carrier proteins and hence not available at the cell receptor level to stimulate fetal growth and trophoblast invasion of the decidua[96]. Many studies have reported reduced maternal serum PAPP-A concentration at 11–14 weeks and increased risk for subsequent development of preeclampsia, IUGR and preterm delivery [97 98 99].

For IUGR, the sensitivity of PAPP-A below 5 th percentile in the first trimester were 8–33% [99].

Our data show that MoM PAPP-A was significantly lower in maternal form, late onset hypertensive diseases and IUGR group.

We think there is an association with metabolic syndrome: Sifakis et al [100] has demonstrated that at 11–13 weeks' gestation in pregnancies destined to develop preeclampsia compared with unaffected controls, the median maternal serum IGFBP-3 and uterine artery PI were higher and serum PAPP-A was lower. Evidence from both in vitro and in vivo studies suggested that increased levels of IGFBP-3 are associated with hyperglycemia, metabolic syndrome and increased insulin resistance[101].

In women destined to develop late hypertensive disorders, placental perfusion and fetal growth are often normal and the main pathophysiological processes resemble those of the metabolic syndrome with an increase in adipose tissue, impaired glucose tolerance and increased insulin resistance[102 103]. In non-pregnant women, insulin is the main

regulator of IGFBP-1, and studies in pregnancy reported that IGFBP-1 production by the decidualized stroma is inhibited by insulin[104]. Always in 2011, Sifakis et al[105] wrote that the low levels of IGFBP-1 in women destined to develop preeclampsia may be the consequence of the associated hyperinsulinemia and increased insulin resistance[106].

Maternal factors

Among the maternal factors that may contribute to the development of maternal hypertensive disorders, body mass index seems to play an important role. In our study maternal form showed a higher values of BMI than placental ones. This factor is well related to metabolic syndrome, low grade chronic inflammation and insulin resistance and it may be correlated with the physiopathology of the disease. Fat tissue is metabolically active, producing numerous inflammatory mediators (leptin, adiponectin, interleukins (IL)-1, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), interferon-a and monocyte chemoattractant protein (MCP)-1. Adipokines are secreted into the systemic circulation and represent a trigger point to activate the inflammation cascade. All this process that is fundamental in maternal hypertensive disorders, could influence also placental form, worsening and enhancing the inflammation process typical of hypertensive disease.

Conclusion

Considering the seriousness of placental form and its impact on the fetus, the use of a classification allowing to recognize different manifestations of the disease could be the first step towards a reliable preventive practice. The classical temporal definition of hypertensive disease may underestimate the risk, leading to a failure of screening and prevention. This change of classification, not based on gestational age anymore but on the aetiology of the disease, allows us to carefully evaluate the risk of fetal distress and additional problems. The most important goal is to identify placental hypertensive disorders that are associated with the highest perinatal and maternal morbidity and mortality.

This model is a potentially helpful tool to identify the highest risk group of patients that may develop important complications. Even more, the possibility of giving a prediction rate with good specificity in one step could be important in some health situations where the follow-up of the patients could be difficult.

The ability to predict in very early pregnancy those women at risk for PE might decrease maternal and fetal morbidity through closer surveillance by physicians experienced or specialized in high-risk obstetrics, as well as delivery at tertiary care centres[107]. Centralized care of pregnancies at high risk for hypertensive disorders would also lead to a more effective concentration of research activity in an attempt to improve the understanding of the pathophysiology and treatment of the condition.

In conclusion, the screening test proposed with the new aetiological classification, seems to be very effective in prediction of placental hypertensive disorders, and in discrimination in maternal hypertensive disorders.

We have demonstrated that, classifying hypertensive disorders according to aetiological criteria, the predictive power increases, allowing to identify a larger number of pregnant women at risk of developing a hypertensive disorder.

Early identification of women at risk is very important, especially in cases of placental form, because this disease is responsible for serious maternal and fetal consequences. The prevention is a fundamental resource, and screening for this pathology would change the history of the disease.

Many studies are still needed, both to understand the aetiology of these diseases and for identifying a cure, but our work could improve clinical management of these patients.

Limits of the study

We acknowledge that the number of cases in the study is too small (the prevalence of HD in our cohort is low) to draw firm conclusions, and confirmation from larger studies will be required. Smoking habit was not considered. Diagnosis of early and late preeclampsia is based on time at delivery and not at time of diagnosis.

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